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PRINCIPAL INVESTIGATOR: Stephen D. Walker

CONTRACTING ORGANIZATION: Vixel Corporation
Broomfield, Colorado 80021

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This Small Business Innovation Research Phase I project proved the feasibility of a compact and low cost instrument to measure tissue oxygenation based on near-infrared (NIR) spectroscopy. NIR light in the .7-1.1 µm range penetrates human tissue to a depth of several centimeters, and is attenuated during transmission by oxyhemoglobin, deoxyhemoglobin, melanin, water and cytochrome aa3, each with its own characteristic absorption spectrum. NIR absorption spectroscopy offers a number of advantages in comparison with other oxygen measurement modalities: it is non-invasive, uses non-ionizing radiation, offers high spatial and temporal resolution, and supplies new types of metabolic and functional information for low cost, rapid, bedside diagnostic procedures. The Phase I project determined the most practical means to implement a hand-held NIR spectroscopic oxygenation monitor, specified the components needed, defined the measurement method and assessed the practicality of the instrument for Phase II development.

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Non Invasive Deep Tissue Oxygenation Sensor

Phase I Final Report

INTRODUCTION

The aim of this Phase I project was to assess the practicality of using laser diodes specifically for tissue oxygenation near infrared spectroscopy. Near Infrared (NIR) light penetrates human tissue to a depth of several centimeters. Light is attenuated during transmission by dominant absorbers, each of which absorbs at a specific wavelength in the NIR region. These dominant absorbers are involved with cellular metabolism and tissue oxygenation. The amount of light absorbed is proportional to the concentration of the absorber. Figure 1 illustrates the basic principle.

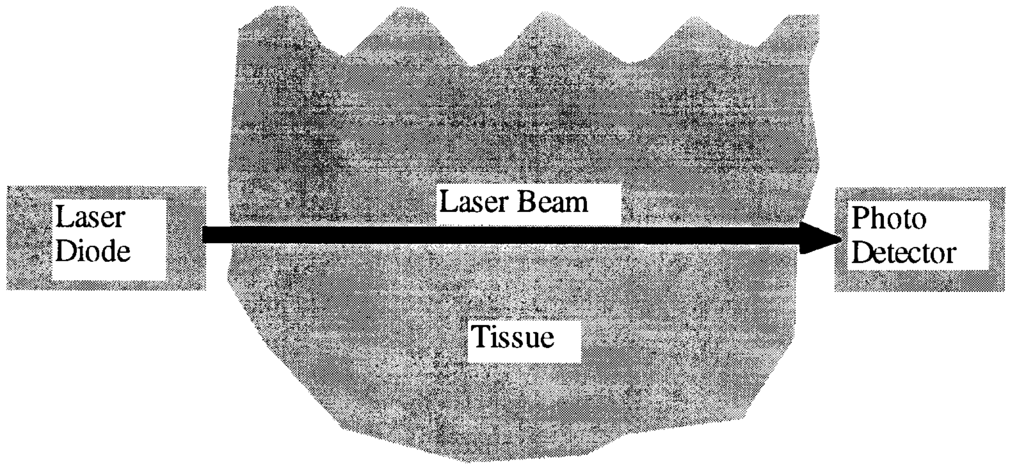

Figure 1. NIR laser light is directed through tissue onto a photodetector. For a specific wavelength, the amount of light absorbed is proportional to the amount of absorber in the optical path.

The project developed a multiple discrete wavelength approach to perform NIR spectroscopy. First, we determined the absorption spectra of absorbers that are involved in tissue oxygenation. Potential absorbers were oxyhemoglobin, deoxyhemoglobin, metoxyhemoglobin, carboxyhemoglobin, cytochrome aa3, melanin, and water. By using two discrete lasers with emission wavelengths corresponding to absorptive bands of two different absorbers, the amounts of the two different absorbers can be measured. Amounts of four different absorbers can be determined with four different emission wavelengths.

Conventional edge-emitting laser diode are prevalent in commercial products such as compact disk players, desktop laser printers, and handheld barcode scanners. Vertical-Cavity Surface-Emitting Lasers (VCSELs) are an emerging form of laser diode which have intrinsic characteristics far superior to edge-emitters in terms of manufacturing, compact low-cost packaging, and spectral stability. While edge emitters are several hundred microns long and emit in the plane of the chip, VCSELs are circular, about 10 µm in diameter, and emit perpendicular to the plane of the chip, providing very easy access to the beam. VCSELs are manufactured by standard GaAs processing techniques and the emitted beams are circular, low-divergence and aberration free. The fabricated VCSEL is a nearly-planar structure which can be tested while still on the wafer by completely automated means, thereby greatly reducing the cost of the laser. Required operating currents are only 3-5 milliamps, compared to 30-50 milliamps in a compact-disk laser. Thus the power required for the VCSEL-based sensor will be much lower than an edge-emitter based one, which is an important consideration for hand-held, battery-operated instruments utilizing multiple lasers.

Non Invasive Deep Tissue Oxygenation Sensor
Phase I Final Report

BODY

Phase I Technical Objectives

1. Analyze spectra of primary absorbers.
2. Determine the optimum set of wavelengths for monitoring oxygenation.
3. Define the measurement method for measuring oxygenation.
4. Assess the overall practicality of the proposed instrument.
5. Report results and develop a Phase II plan to develop a prototype to be commercialized in a Phase III program.

Analyze spectra of primary absorbers

1. Oxyhemoglobin. Oxyhemoglobin is oxygenated hemoglobin and equivalent to arterial blood. Knowledge of oxyhemoglobin concentration is essential for the measurement of tissue oxygenation because it is the primary oxygen source. Low concentrations of oxyhemoglobin are caused by insufficient oxygenation of the blood in the pulmonary system. This can be the result of insufficient cardiac output or improper ventilation. Localized high concentrations are an indicator of hematomas or necrotic tissue because cellular metabolism is not taking place. Oxyhemoglobin has a broad absorption band from 600-810 nm.
2. Deoxyhemoglobin. Deoxyhemoglobin is the reduced hemoglobin molecule and equivalent to venous blood. Low concentrations of deoxyhemoglobin indicate cellular metabolism is not taking place or the hemoglobin molecule is immediately combining with interstitial oxygen. Deoxyhemoglobin has a broad absorption band from 800-1000 nm.
3. Cytochrome aa3. Cytochrome aa3 is a mitochrondrial enzyme whose presence indicates cellular metabolism is taking place. It has a moderate absorption band from 830-870 nm.
4. Melanin. Melanin is a skin pigment and essentially an interfering absorber. Different skin colors absorb NIR light to different degrees. It has a broad absorption band from 600-1000 nm.
5. Water. Water is everywhere and has several very high absorption bands. These bands are specified in the HITRAN 92 database.

Potential interfering NIR absorbers found in tissue were also identified and are shown in Figure 2.

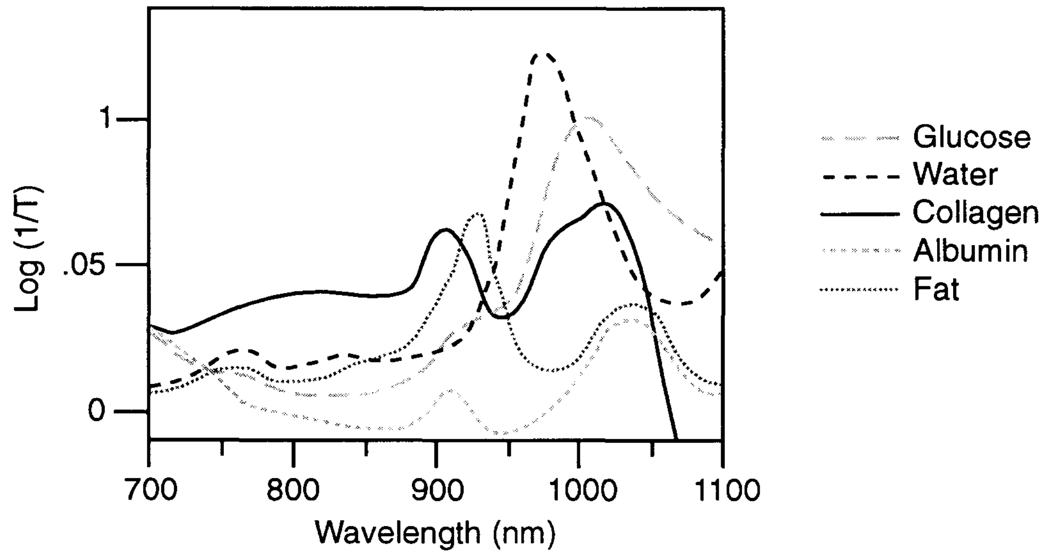

Figure 2. Near IR absorption spectra of glucose and interfering absorbers.

Determine the optimum set of wavelengths for monitoring oxygenation

Neuro Shell 2, a commercial neural network program, was used to develop a software model of the proposed spectrometer. The objective of the simulation was to identify five laser diode wavelengths which when used as tissue oxygenation spectrometer light sources could quantify the targeted absorbers. Fabricating laser diodes at a specified wavelength is very expensive and the advantage of this simulator is obvious. This simulation ran for 22 hours on a 25 MHz 486 computer and selected 5 wavelengths shown in Figure 3.

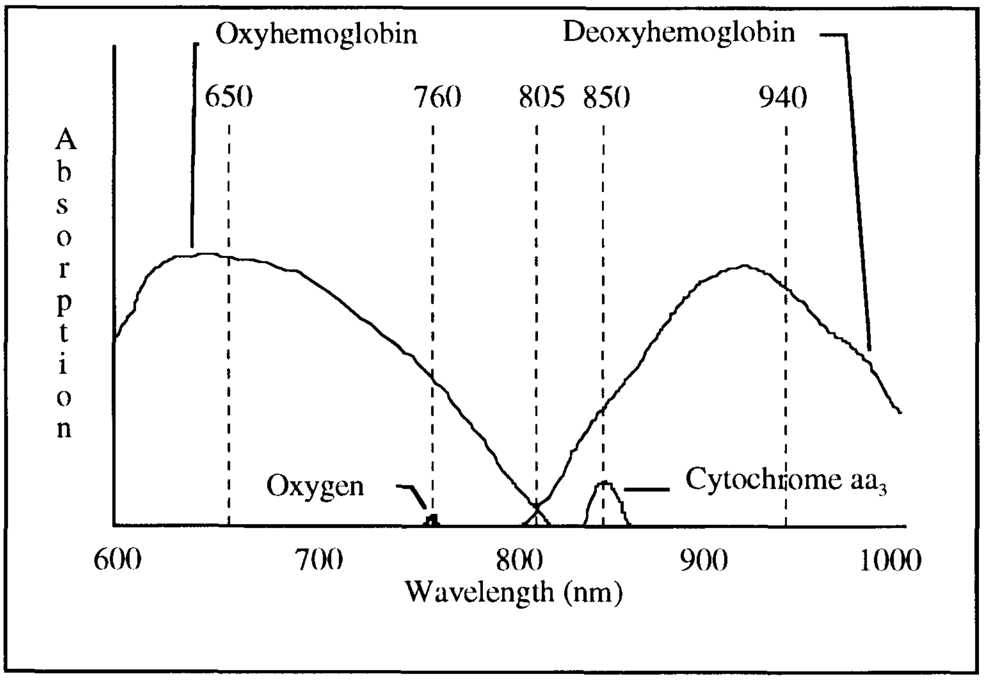

Figure 3. NIR absorption spectra of oxyhemoglobin, deoxyhemoglobin, oxygen and cytochrome aa3. Laser diode wavelengths selected by simulation are shown as dotted lines.

The simulator was first trained to recognize the primary absorbers and oxygen by giving it their individual spectra as inputs. It was then trained to recognize combinations of 2 spectra. Finally, it was allowed to choose only five wavelengths in the spectra as inputs. The simulator varied these five wavelengths until it found the highest recognition rate. Melanin and water spectra were not simulated because they are not directly associated with cellular metabolism.

Define the measurement method for measuring oxygenation

A prototype spectrometer was constructed and tested on the oxygen wavelength to validate the WMS method. The block diagram is shown in Figure 4. A thermoelectric cooler is used to adjust the VCSEL temperature for an emitted wavelength of 760.3 nm. Wavelength modulation of 0.2 nm is achieved by applying a sawtooth drive current to the VCSEL. The laser beam is directed onto a beamsplitter where 50% of the energy is reflected through a reference cell containing pure nitrogen and onto a detector whose output is the inverting input to a differential amplifier. The 50% of the energy which passed through the beamsplitter is reflected off a mirror through the sample chamber containing pure oxygen and onto a detector whose output is the non-inverting input to the differential amplifier. The output of the amplifier is the oxygen spectra.

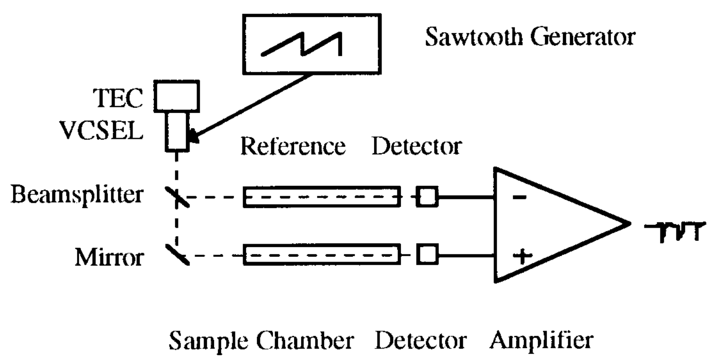

Figure 4. Block diagram of the prototype Wavelength Modulated Spectrometer.

Figure A1, in the appendix, shows oscilloscope waveforms from the prototype spectrometer. The resolution shown compares favorably with FTIR spectrometers and laboratory Laser Spectrometers at a fraction of the cost and size.

The oxygen absorption spectrum in the 761 nm region is shown in Figure 5. Individual oxygen resonance linewidths are on the order of 1 GHz. VCSEL's have 65 MHz linewidth [Olbright, 1992], which is much smaller than an oxygen resonance. The VCSEL's narrow beam is swept from outside to inside and back outside the oxygen resonance by varying the emission wavelength by varying the VCSEL temperature with a sawtooth waveform. The differential amplitude of the sidebands is zero outside the resonance and significant inside the resonance. How significant depends on the slope of the resonance.

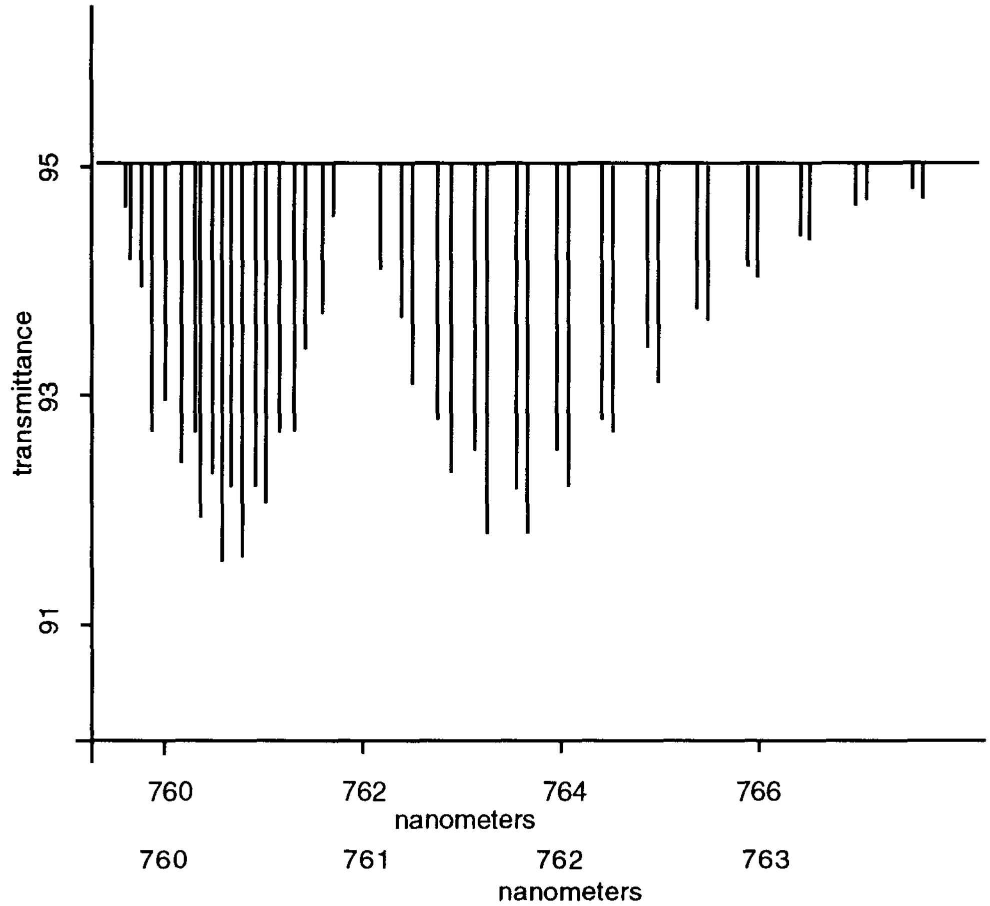

Figure 5. Oxygen absorption spectrum. The laser diode has an emission wavelength within this region and is tuned to an absorption line by varying the VCSEL temperature.

The percentage of light absorbed can be very small when sensing gases dissolved in interstitial fluid. This necessitates the use of differential and self-referencing techniques. A particularly successful technique which is well-suited for laser diodes is wavelength modulation in which a laser diode is modulated at over 100 MHz by modulations in its current supply produced by a crystal oscillator. The modulation produces sidebands in the spectral emission as shown in Figure 6. The sidebands are separated from the optical carrier frequency by integral multiples of the modulation frequency. Under weak modulation, the laser spectrum can be approximated by the carrier frequency, ω_o, and single upper and lower sidebands at ω_o - ω_m and ω_o + ω_m [see Figure 6(a)]. When light with this spectrum is detected in a square-law photodiode, each of the sidebands mixes with the carrier to generate a signal at the modulation frequency. These two signals are equal in amplitude and 180° out of phase, thus they exactly cancel each other out. If one of the sidebands is absorbed prior to detection [see Figure 6(b)], the amplitudes are no longer equal [see Figure 6(c)] and a signal at the modulation frequency ω_m appears in the detector current. The amplitude of this signal is directly proportional to the amount of light absorbed. By filtering the detector output at the modulation frequency ω_m, the absorption is obtained. Tunability of the laser diode wavelength tunes all three emission wavelengths together. Tuning such that one of the sidebands tunes throughout the absorption line is useful for two main reasons: 1) one insures that the maximum absorption is measured; and 2) the shape of the absorption line can be traced. For the oxygen sensor, this provides an absolute measure of the amount of oxygen in the path of the

laser beam. Because the carrier and the sidebands traverse exactly the same optical path, the technique is self-referencing. Since the sideband signals cancel each other out in the absence of differential absorption of one sideband with respect to the other, the technique is inherently differential. Excellent sensitivity of 10^{-4} is therefore obtained.

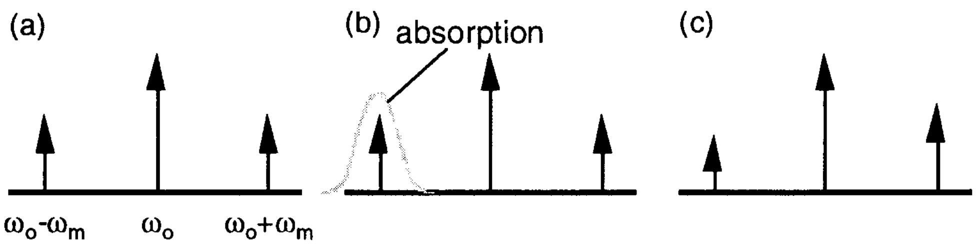

Figure 6. Frequencies of light emitted by a modulated laser diode under WM operation. (a) Carrier frequency and equal-amplitude sidebands emitted. (b) Presence of absorption at the lower sideband frequency. (c) Resultant inequality in the sideband amplitudes.

Assess the overall practicality of the proposed instrument

The goal of the Phase I project was to establish the feasibility of a low-cost, handheld instrument for noninvasive measurement of tissue oxygenation. Spectral analysis of oxyhemoglobin, deoxyhemoglobin, cytochrome aa3, melanin and water showed oxyhemoglobin, deoxyhemoglobin and cytochrome aa3 have useful spectral features. Melanin and water are essentially constants and are not directly involved in cellular metabolism. Oxygen was added to the list of targeted absorbers because it has useful spectral features and are directly involved in tissue oxygenation. Simulation of the proposed instrument indicated 5 VCSEL wavelengths which could resolve levels of oxyhemoglobin, deoxyhemoglobin, cytochrome aa3 and oxygen. The feasibility of using Wavelength Modulated Spectroscopy for the measurement method was established with a prototype spectrometer for oxygen.

CONCLUSIONS

The proposed instrument for tissue oxygenation is practical. The targeted absorbers have been shown to have absorbances within the range of VCSEL technology. VCSELs are low cost semiconductors with the necessary power, sensitivity and resolution to penetrate a dense scattering media like tissue. The measurement method chosen, WMS, is easily implemented with off-the-shelf components and does not involve any complex, high frequency circuitry.

Three additional instruments based on this technology were proven to be practical during the Phase I project. Bioreactors have a need for low-cost, rapid noninvasive measurement of oxygen and glucose sensors. A noninvasive instrument for oxygen blood gas measurement based on the bioreactor oxygen sensor is practical as is a noninvasive blood sugar meter based on the bioreactor glucose sensor.

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APPENDIX

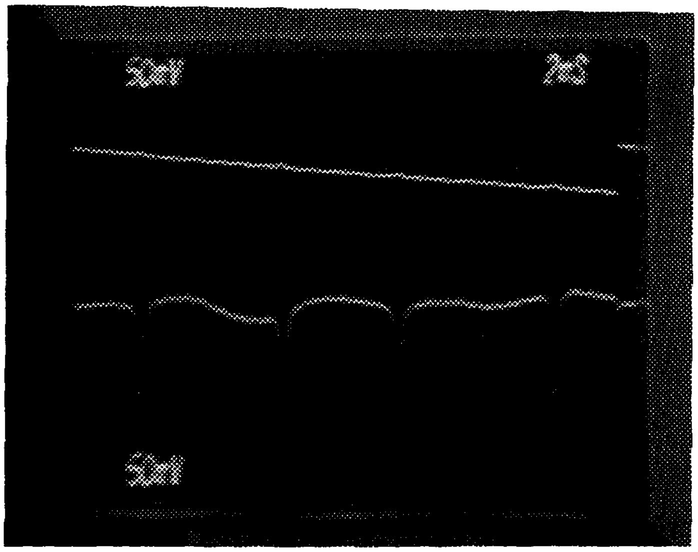

Figure A1. Photograph of VCSEL drive current (top) and oxygen spectra (bottom).

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